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(54) Title: USE OF ASTAXANTHIN FOR RETARDING AND AMELIORATING CENTRAL NERVOUS SYSTEM AND EYE DAMAGE

(57) Abstract

A method of retarding and ameliorating eye diseases and injuries is disclosed. The method comprises administering astaxanthin in a therapeutically-effective amount to prevent, retard or treat eye and central nervous system diseases or injuries, such as age-related macular degeneration and other central nervous system degenerative diseases, photic injury, ischemic diseases, and inflammatory diseases.

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USE OF ASTAXANTHIN FOR RETARDING AND AMELIORATING CENTRAL NERVOUS SYSTEM AND EYE DAMAGE

FIELD OF THE INVENTION

The present invention relates to a method of
5   retarding and ameliorating central nervous system and
eye diseases. More particularly, the present inven-
tion is directed to methods of treating central
nervous system and eye insult resulting from disease
or injury, such as age-related macular degeneration,
10   photic injury, photoreceptor cell or ganglion cell
damage, traumatic injury, ischemic insult-related
diseases and inflammatory diseases. The method
comprises administering a therapeutically-effective
amount of astaxanthin to an individual, either orally,
15   topically or parenterally, to ameliorate damage caused
by the disease or injury or to retard the progress of
a degenerative disease.

BACKGROUND OF THE INVENTION

Many diseases and injuries of the central
20   nervous system presently are not treatable. These
diseases and injuries are untreatable because, unlike
many peripheral organs that can be removed in whole or
in part, or transplanted, the central nervous system
has very limited regeneration capability and cannot be
25   totally excised without death. The eye is an exten-
sion of the brain, and therefore a part of the central
nervous system. Accordingly, in the case of an eye
injury or disease, i.e., a retinal injury or disease,
the diseases are often without treatment and the eye
30   cannot be transplanted. Eye diseases and injuries
that presently are untreatable include retinal photic
injury, retinal ischemia-induced eye injury, age-

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related macular degeneration, and other eye diseases and injuries that are induced by free radical species.

It has been hypothesized that a major cause of these untreatable central nervous system diseases and injuries is the generation and presence of singlet oxygen and other free radical species. Singlet oxygen and free radical species can be generated by a combination of light and oxygen, or during reperfusion after an ischemic insult.

The eye is subjected to continuous light exposure because the primary purpose of the eye is light perception. Therefore, some untreatable diseases and injuries to the eye result from the continuous exposure of the eye to light, coupled with the highly-oxygenated environment in the eye.

The process of light perception is initiated in the photoreceptor cells. The photoreceptor cells are a constituent of the outer neuronal layer of the retina, which is a component of the central nervous system. The photoreceptor cells are well sheltered in the center of the eye, and are protected structurally by the sclera, nourished by the highly-vascularized uvea and safeguarded by the blood-retinal barrier of the retinal pigment epithelium.

The primary function of the photoreceptor cells is to convert light into a physio-chemical signal (transduction) and to transmit this signal to the other neurons (transmission). During the transduction and transmission processes, the metabolic activities of these neurons are changed dramatically. Even though the photoreceptor cells are securely protected in the interior of the eye, these cells are readily accessible to light because their primary function is light detection. Excessive light energy

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reaching the retina can cause damage to these neurons, either directly or indirectly, by overwhelming the metabolic systems of these cells.

The combination of continuous and/or excessive exposure to light, and the relatively high concentration of oxygen in the eye, generates singlet oxygen and other free radical species. Singlet oxygen and free radical species also can be generated by enzymatic processes independent from light exposure. The free radical species and singlet oxygen are reactive entities that can oxidize polyunsaturated fatty acids. The retina contains the highest concentration of polyunsaturated fatty acids of any tissue in the human body, and peroxidation of the polyunsaturated fatty acids in cell membranes of the retina by hydroxyl radicals (OH$\cdot$) or superoxide (O$_2\cdot$) radicals can propagate additional free radical species. These free radical species can lead to functional impairment of the cell membranes and cause temporary or permanent damage to retinal tissue. It has been theorized that the generation of singlet oxygen and free radical species therefore underlies the pathogenesis of light-induced retinopathy and post-ischemic reflow injury (i.e., free radical generation during reperfusion after an ischemic insult). In addition, a deficiency in removing these species can also contribute to various diseases of the eye and the central nervous system.

A number of natural mechanisms protect the photoreceptor cells from light injury. For example, the ocular media, including the cornea, aqueous, lens, and vitreous, filter most of the light in the ultraviolet region. However, after cataract extraction or other surgical intervention, some of these protective

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barriers are removed or disturbed, whereby the photo-
receptor cells are more susceptible to damage by
radiant energy. The photoreceptor cells also possess
other forms of protection from photic injury, for
5   example, the presence of antioxidant compounds to
counteract the free radical species generated by
light. As will be demonstrated hereafter, antioxi-
dants, which quench and/or scavenge singlet oxygen,
hydrogen peroxide, superoxide and radical species,
10   help minimize injury to the photoreceptor cells. In
addition, the human eye has an excessive number of
photoreceptor cells such that only destruction of a
significant number of photoreceptor cells adversely
affects visual function.

15   Even though several protective mechanisms
are present in the eye, a leading cause of blindness
in the United States is age-related photoreceptor
degeneration. Clinically, photoreceptor degeneration,
as seen in age-related macular degeneration, is
20   causally related to excessive exposure to blue light.
The causes of age-related macular degeneration, which
is characterized by a loss of photoreceptor neurons
resulting in decreased vision, are being investigated.
Epidemiological studies indicate that age-related
25   photoreceptor degeneration, or age-related macular
degeneration, is caused by several factors including
age, sex, family history, color of the iris, nutri-
tional deficiency, immunologic disorders, cardiovascu-
lar and respiratory diseases and preexisting eye
30   diseases. Advancing age is the most significant
factor. Recently, it has been demonstrated that aging
eyes have a decreased amount of carotenoids. Clinical
and laboratory studies indicate that photic injury is
a cause of age-related macular degeneration because of

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the cumulative effect of repeated mild photic insult which leads to a gradual loss of photoreceptor cells.

Age-related macular degeneration is an irreversible blinding disease of the retina. Unlike cataract wherein vision can be restored by replacing the diseased lens, age-related macular degeneration cannot be treated by replacing the diseased retina because the retina is a component of the central nervous system. Therefore, because no treatment for this disease exists once the photoreceptors are destroyed, prevention is the only way to confront age-related macular degeneration. Presently, prevention of age-related macular degeneration resides in limiting or preventing light and oxygen-induced (i.e., free radical-induced) damage to the retina because the retina is the only organ that is continuously exposed to high levels of light in a highly-oxygenated environment.

In addition to photic injury, eye injury and disease can result from singlet oxygen and free radical species generated during reperfusion after an ischemic insult. Ischemic insult to retinal ganglion cells and to neurons of the inner layers of retina causes loss of vision. Loss of vision accompanies diabetic retinopathy, retinal arterial occlusion, retinal venous occlusion and glaucoma, each of which ischemically insults the eye, i.e., deprives the eye of oxygen and nutrition.

The damage to the retinal ganglion cells has been attributed to ischemia, and subsequent reperfusion during which free radicals are generated. During reperfusion, the reoxygenation of tissue after an ischemic insult results in a relatively high concentration of oxygen flowing through the effected

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tissue, which contributes to free radical formation. Ischemic insult and reperfusion accompanied by free radical generation also is a major contributor to central nervous system damage, such as damage caused by a stroke.

The pathogenesis of photic injury, of age-related macular degeneration, of ischemia/reperfusion damage, of traumatic injury and of inflammations of the eye and central nervous system have been attributed to singlet oxygen and free radical generation, and subsequent free radical-initiated reactions. Investigators therefore studied the role of antioxidants in preventing or ameliorating these diseases and injuries of the central nervous system in general, and the eye in particular.

For example, ascorbate was investigated as an agent to treat retinal photic injury. Ascorbate is a reducing agent which is present in the retina in a high concentration. Studies indicated that ascorbate in the retina can act as an antioxidant and is oxidized by free radical species generated during excessive light exposure.

Administration of ascorbate reduced the loss of rhodopsin after photic exposure, thereby suggesting that ascorbate offered protection against retinal photic injury. A decrease in rhodopsin levels is an indicator of photic eye injury. The protective effect of ascorbate is dose-dependent, and ascorbate was effective when administered before light exposure. Morphometric studies of the photoreceptor nuclei remaining in the retina after light exposure showed that rats given ascorbate supplements had substantially less retinal damage. Morphologically, rats with

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ascorbate supplements also showed better preservation of retinal pigment epithelium.

The above studies led to the hypothesis that ascorbate mitigates retinal photic injury because of its antioxidant properties, which are attributed to its redox properties. Ascorbate is a scavenger of superoxide radicals and hydroxy radicals and also quenches singlet oxygen and reduces hydrogen peroxide, all of which are formed in retinal photic injury. This hypothesis accounts for the presence of high levels of naturally-occurring ascorbate in a normal retina.

Therefore, antioxidants which inhibit free radical formation, or which quench singlet oxygen and scavenge for free radical species, can decrease lipid peroxidation and ameliorate photic injury and isch-emic/reperfusion injury in the central nervous system, and particularly in the retina. Antioxidants originally were investigated because they are known con-stituents of human tissue. However, antioxidants that are not naturally occurring in human tissue also were tested. In particular, in addition to ascorbate, antioxidants such as 2,6-di-<u>tert</u>-butylphenol, γ-oryzanol, α-tocopherol, mannitol, reduced glutathione, and various carotenoids have been studied for an ability to quench singlet oxygen and scavenge free radical species. These and other antioxidants are effective quenchers and scavengers for singlet oxygen and free radicals. In particular, the carotenoids, as a class of compounds, are very effective singlet oxygen quenchers and free radical scavengers. Howev-er, individual carotenoids differ in their ability to quench singlet oxygen and scavenge for free radical species.

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The carotenoids are naturally-occurring compounds that have antioxidant properties. The carotenoids are common compounds manufactured by plants or animals, and contribute greatly to the coloring of plants and animals. A number of animals, including mammals, are unable to synthesize carotenoids *de novo* and accordingly rely upon diet to provide carotenoid requirements. Mammals also have a limited ability to modify carotenoids. A mammal can convert β-carotene to vitamin A, but most other carotenoids are deposited in mammalian tissue in unchanged form.

With respect to humans, about ten carotenoids are found in human serum. The major carotenoids in human serum are β-carotene, α-carotene, cryptoxanthin, lycopene and lutein. Small amounts of zeaxanthin, phytofluene, and phytoene are found in human organs. However, of the ten carotenoids found in human serum, only two, zeaxanthin and lutein, are found in the human retina. Zeaxanthin is the predominant carotenoid in the macula and is concentrated in the cone cells in the center of the retina, i.e., the macula. Lutein is located in the peripheral retina in the rod cells. Therefore, the eye preferentially assimilates zeaxanthin over lutein in the macula and also selectively concentrates zeaxanthin, which is a more effective singlet oxygen scavenger than lutein, in the center of the eye. It has been theorized that zeaxanthin and lutein are concentrated in the retina because of their ability to quench singlet oxygen and scavenge free radicals, and thereby limit or prevent photic damage to the retina.

Therefore only two of the about ten carotenoids present in human serum are found in the retina.

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Beta-carotene and lycopene, the two most abundant
carotenoids in human serum, either have not been
detected or have been detected only in minor amounts
in the retina. Beta-carotene is relatively inaccessi-
ble to the retina because β-carotene is unable to
cross the blood-retinal brain barrier of the retinal
pigment epithelium effectively. As will be explained
in detail hereinafter, small amounts of β-carotene
have been found to cross the blood-retinal brain
barrier.

It also is known that another carotenoid,
canthaxanthin, can cross the blood-retinal brain
barrier and reach the retina. Canthaxanthin, like all
carotenoids, is a pigment and can discolor the skin.
Canthaxanthin provides a skin color that approximates
a suntan, and accordingly has been used by humans to
generate an artificial suntan. However, an undesir-
able side effect in individuals that ingested
canthaxanthin at high doses for an extended time was
the formation of crystalline canthaxanthin deposits in
the inner layers of the retina. Therefore, the blood-
retinal brain barrier of the retinal pigment epatheli-
um permits only particular carotenoids to enter the
retina. The carotenoids other than zeaxanthin and
lutein that do enter the retina cause adverse effects,
such as the formation of crystalline deposits by
canthaxanthin, which may take several years to dis-
solve. Canthaxanthin in the retina also caused a
decreased adaptation to the dark.

Investigators have unsuccessfully sought an
antioxidant to counteract the adverse affects of
singlet oxygen and free radical species on the central
nervous system in general and the eye in particular.
The investigators have studied the antioxidant capa-

bilities of several compounds, including various carotenoids. Even though the carotenoids are strong antioxidants, investigators have failed to find particular carotenoids among the 600 naturally-occur-
5   ring carotenoids that effectively quench singlet oxygen and scavenge for free radical species, that are capable of crossing the blood-retinal brain barrier, that do not exhibit the adverse affects of canthaxanthin after crossing the blood-retinal brain
10 barrier, and that ameliorate central nervous system or eye disease or injury and/or retard the progression of a degenerative disease of the central nervous system or eye.

Various publications are directed to eye
15 diseases and injuries, such as age-related macular degeneration, causes of the damage resulting from the diseases or injuries, and attempts to prevent or treat such diseases and injuries. The publications, which discuss various antioxidants, including the carote-
20 noids and other antioxidants like α-tocopherol, include:

M.O.M. Tso, "Experiments on Visual Cells by Nature and Man: In Search of Treatment for Photore-ceptor Degeneration", Investigative Ophthalmology and
25 Visual Science, 30(12), pp. 2421-2454 (December, 1989);

W. Schalch, "Carotenoids in the Retina-A Review of Their Possible Role in Preventing or Limit-ing Damage Caused by Light and Oxygen", Free Radicals
30 and Aging, I. Emerit et al. (ed.), Birkhauser Verlag, pp. 280-298 (1992);

M.O.M. Tso, "Pathogenetic Factors of Aging Macular Degeneration", Ophthalmology, 92(5), pp. 628-635 (1985);

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M. Mathews-Roth, "Recent Progress in the Medical Applications of Carotenoids", Pure and Appl. Chem., 63(1), pp. 147-156 (1991);

W. Miki, "Biological Functions and Activities of Animal Carotenoids", Pure and Appl. Chem., 63(1), pp. 141-146 (1991);

M. Mathews-Roth, "Carotenoids and Cancer Prevention-Experimental and Epidemiological Studies", Pure and Appl. Chem., 57(5), pp. 717-722 (1985);

M. Mathews-Roth, "Porphyrin Photosensitization and Carotenoid Protection in Mice; In Vitro and In Vivo Studies", Photochemistry and Photobiology, 40(1), pp. 63-67 (1984);

P. DiMascio et al., "Carotenoids, Tocopherols and Thiols as Biological Singlet Molecular Oxygen Quenchers", Biochemical Society Transactions, 18, pp. 1054-1056 (1990);

T. Hiramitsu et al., "Preventative Effect of Antioxidants on Lipid Peroxidation in the Retina", Ophthalmic Res., 23, pp. 196-203 (1991);

K. Kirschfeld, "Carotenoid Pigments: Their Possible Role in Protecting Against Photooxidation in Eyes and Photoreceptor Cells", Proc. R. Soc. Lond., B216, pp. 71-85 (1982);

D. Yu et al., "Amelioration of Retinal Photic Injury by Beta-Carotene", ARVO Abstracts Invest. Ophthalmol. Vis. Sci., 28 (Suppl.), p. 7, (1987);

M. Kurashige et al., "Inhibition of Oxidative Injury of Biological Membranes by Astaxanthin", Physiol. Chem. Phys. and Med. NMR, 22, pp. 27-38 (1990); and

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N.I. Krinsky et al., "Interaction of Oxygen and Oxy-radicals With Carotenoids", _J. Natl. Cancer Inst._, _69(1)_, pp. 205-210 (1982).

In general, the above-identified publications support
5   the hypothesis that singlet oxygen and free radical species are significant contributors to central nervous system, and particularly eye, injury and disease. For example, it has reported that consumption of an antioxidant, such as ascorbic acid (Vitamin
10   C), α-tocopherol (Vitamin E) or β-carotene, can decrease the prevalence of age-related macular degeneration.
The above-identified publications also demonstrated that several carotenoids, including
15   astaxanthin, are strong antioxidants compared to β-carotene, ascorbic acid and other widely used antioxidants. The publications also relate that (1) only particular carotenoids selectively cross the blood-retinal brain barrier, and that (2) certain carote-
20   noids other than zeaxanthin and lutein that cross the blood-retinal brain barrier cause adverse affects.
In general, the above-identified publications teach that astaxanthin is a more effective antioxidant than carotenoids such as zeaxanthin,
25   lutein, tunaxanthin, canthaxanthin and β-carotene, and than α-tocopherol. For example, the _in vitro_ and _in vivo_ studies disclosed in the Kurashige et al. publication with respect to astaxanthin demonstrated that the mean effective concentration of astaxanthin which
30   inhibits lipid peroxidation was 500 times lower than that of α-tocopherol. Similarly, the Miki publication discloses that, _in vitro_, astaxanthin exhibits a strong quenching effect against singlet oxygen and a

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strong scavenging effect against free radical species.

        Investigators have theorized that free
radical-mediated reactions are involved in the patho-
5   genesis of degenerative diseases, such as Alzheimer's
disease, Parkinson's disease and age-related macular
degeneration, ischemic/reperfusion damage and traumat-
ic injury of the brain, spinal cord and retina, as
well as in inflammatory processes of the body, and
10   particularly the eye. This theory has been advanced
by investigators examining the effectiveness of
various antioxidants in ameliorating these diseases.
For example, methylprednisolone, which at high doses
is a strong lipid peroxidation inhibitor, has been
15   recommended clinically for use in traumatic injury of
the spinal cord.
        To date, investigative efforts have been
directed to preventing diseases and injury because the
resulting free radical-induced damage is not effec-
20   tively treatable. Therefore, a need exists for a
method not only to prevent or retard, but also to
ameliorate, degenerative and traumatic diseases and
injuries to the central nervous system, and particu-
larly the eye. The present invention is directed to
25   such methods.

                  SUMMARY OF THE INVENTION
        The present invention is directed to methods
of treating individuals suffering from central nervous
system injury or disease. More particularly, the
30   present invention is directed to methods of treating
individuals suffering from an eye injury or disease,
and to methods of retarding a degenerative disease of
the eye.

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The method comprises administering a therapeutically-effective amount of astaxanthin to an individual to retard a degenerative disease, or to ameliorate damage to the central nervous system caused
5   by a disease or an injury. In particular, the method comprises administering a therapeutically-effective amount of astaxanthin to an individual to beneficiate the vision of an individual suffering from eye damage caused by disease or injury. The astaxanthin can be
10 administered parenterally, orally or topically.

The method is used to treat free radical-induced eye damage, light-induced eye damage, photoreceptor cell damage, ganglion cell damage, damage to neurons of inner retinal layers, and age-related
15 macular degeneration. The present method also ameliorates neuronal damage to the retina, wherein the neuronal damage is a result of photic injury, or ischemic, inflammatory or degenerative insult.

One aspect of the present invention is to
20 administer about 5 to about 500 milligrams (mg) of astaxanthin, and preferably about 10 to about 200 mg, per kilogram (kg) of body weight per dose, to retard a degenerative disease of the central nervous system or the eye, or to ameliorate damage resulting from an
25 injury or a disease of the central nervous system or the eye.

Another aspect of the present invention is to provide a method of treating an inflammatory disease of the eye by administering a therapeutically-
30 effective amount of astaxanthin to an individual.

Another aspect of the present invention is to treat diseases and injuries to the central nervous system by administering a therapeutically-effective amount of astaxanthin to an individual. The method is

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used to treat diseases and injuries effecting the brain, eye and spinal cord, such as injury caused by spinal cord trauma or by a stroke or neurodegenerative diseases.

5   These and other novel features and aspects of the present invention will become apparent from the following detailed description of the preferred embodiments taken in conjunction with the figures.

BRIEF DESCRIPTION OF THE DRAWINGS

10   FIG. 1 is a bar graph showing the thickness of the ONL (outer nuclear layer) of the retina in micrometers (μm) after light-induced receptor degeneration, and comparing the retinas of control animals to the retinas of animals treated with astaxanthin given

15   parenterally both for the retina as a whole (average) and for each of the four quadrants of the retina;

FIG. 2 is a bar graph showing the thickness of the inner retinal layer (IRL) in micrometers comparing the retinas of control animals to the

20   retinas of animals after ischemic insult treated with astaxanthin;

FIG. 3 is a plot of rhodopsin level vs. time after photic injury comparing the retinas of animals treated orally with astaxanthin to the retinas of

25   control animals; and

FIG. 4 is a plot of ONL thickness vs. time after photic injury comparing the retinas of animals treated orally with astaxanthin to the retinas of control animals.

30   DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Certain diseases and injuries to the central nervous system, and particularly the eyes, presently

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are untreatable. Neuronal degeneration of the central nervous system or the eye can result from photic injury, ischemic or intraocular pressure-related insult, e.g., an occlusion or a stroke, or from a
5 trauma, e.g., trauma to the spinal cord. The damage also can result from injury to the photoreceptor cells, to the ganglion cells in the retina of the eye or to neurons in the inner retinal layers of the eye, or from age-related macular degeneration.
10   It has been theorized that the damage from such diseases and injuries can be attributed to increased generation, or decreased removal, of singlet oxygen and free radical species. Therefore, because antioxidants are known to quench singlet oxygen and to
15 scavenge for free radical species, and because antioxidants are known to exist in humans, investigators have sought effective antioxidants to scavenge free radicals, and thereby reduce the free radical-induced damage caused to the central nervous system, and
20 especially to the eye, by disease and injury.
        Numerous antioxidants have been investigated, such as for example, ascorbic acid, α-tocopherol and β-carotene. Some investigators have focused on the carotenoids, e.g., β-carotene, because over 600
25 carotenoids are known, are naturally occurring (and therefore are abundant), and are strong antioxidants. Also, with respect to the eyes, two carotenoids, zeaxanthin, and lutein, which are strong antioxidants, are found in the photoreceptor cells of the retina.
30 However, although human plasma includes about ten carotenoids, only these two carotenoids are able to effectively cross the blood-retinal brain barrier and concentrate in the macula of the eye. Beta-carotene, the most abundant human plasma carotenoid, has a very

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limited ability to cross the blood-retinal brain barrier.

The ability of a carotenoid to pass the blood-retinal brain barrier is important because
5   carotenoids are not synthesized by the human body. The only source of carotenoids for humans is dietary intake.   Furthermore, humans have a very limited ability to modify carotenoids. Therefore, the carotenoids accumulate in various organs in the ingested
10 form.   Accordingly, if a particular carotenoid is unable to cross the blood-retinal brain barrier, the carotenoid cannot accumulate in the retina and serve as an antioxidant.

Furthermore, carotenoids that are not normal
15 constituents of human plasma, but have an ability to cross the blood-retinal brain barrier, have demonstrated adverse affects on the retina. Canthaxanthin which is intentionally ingested to provide an artificial suntan has accumulated in the retina in the form
20 of crystals and has temporarily affected eye adaptation to the dark.   In addition, β-carotene has a limited ability to cross the blood-retinal brain barrier.

Therefore, even though the carotenoids are
25 known as strong antioxidants and are present in abundant supply, the carotenoids have not been used for the treatment of central nervous system damage, or eye damage, caused by disease or injury. The carotenoids investigated to date either could not effective-
30 ly cross the blood-retinal barrier (i.e., β-carotene) or adversely affected the eye (i.e., canthaxanthin.

In accordance with an important feature of the present invention, astaxanthin, which is a naturally- occurring compound and is a potent antioxidant,

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is used in a method to ameliorate and retard, or prevent, cell damage in an individual suffering from a degenerative, inflammatory or traumatic disease or injury to the central nervous system, and particularly to the eye. In accordance with another important feature of the present invention, the administration of a therapeutically-effective amount of astaxanthin to an individual prevents, retards and/or ameliorates free radical-induced damage resulting from disease or injury, such as a trauma. For example, damage to a retina can result from either photic injury, neurodegenerative disease or an ischemic insult followed by reperfusion. With respect to damage from photic injury, astaxanthin decreases the loss of photoreceptor cells. With respect to damage from ischemic insult, astaxanthin ameliorates the loss of ganglion cells and the inner layers of the retinal neuronal network.

In general, the carotenoids are terpenoid compounds which are widely-distributed in nature, and which selectively absorb light. About 600 carotenoids have been isolated and identified from various vegetable and animal sources. Animals cannot synthesize carotenoids _de novo_, but rely upon plant sources. Therefore, animals rely on dietary intake for carotenoids. Animals however can modify carotenoids. The ability to modify carotenoids is very limited in humans.

Carotenoids are classified into two major groups: (1) the carotenes, which are hydrocarbons, and (2) the xanthophylls, which include oxygen in addition to carbon and hydrogen. The xanthophylls can be considered oxidation products of the carotenes, and are prepared by the insertion of oxygen into carotenes

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and subsequent rearrangements. The most common carotenoid is the carotene, β-carotene. The following illustrates the chemical structure of various carotenoids:

β-Carotene

Zeaxanthin

Lutein

Canthaxanthin

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Astaxanthin
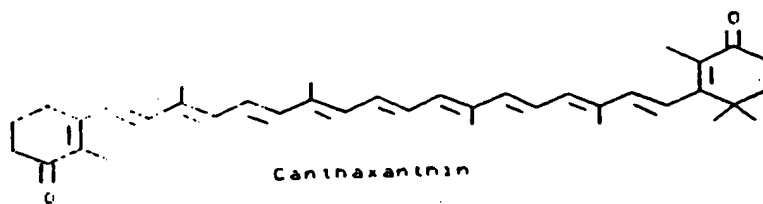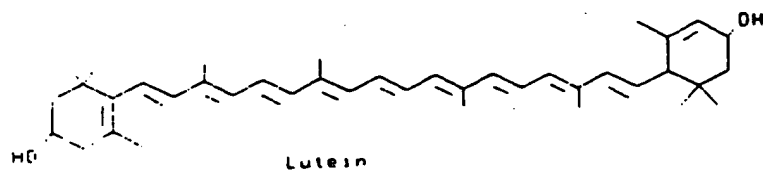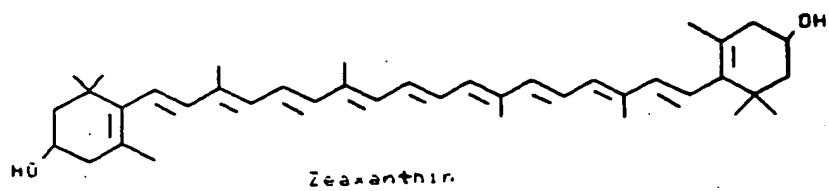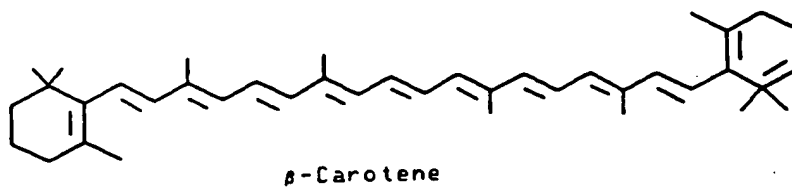
The chemical structures of the five above carotenoids illustrate that minor changes in chemical structure greatly affect the properties of the carotenoid, such as the ability of the carotenoid to cross the blood-retinal brain barrier and the ability of the carotenoid to act as an antioxidant. For example, β-carotene is t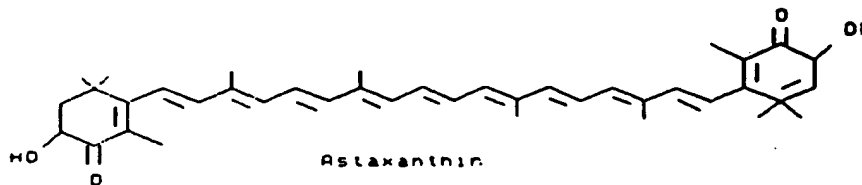he most abundant carotenoid in human plasma, but typically is not found in the retina. Small amounts of β-carotene are found in the retina if β-carotene is administered in high doses intraperitoneally or orally. These results indicate that β-carotene has great difficulty in crossing the blood-retinal brain barrier. In contrast, zeaxanthin and lutein, both of which are very similar in structure to β-carotene, are concentrated in the retina even though zeaxanthin and lutein are present in only minor amounts in human plasma.

With respect to an ability to act as an antioxidant, the carotenoids differ greatly with respect to an ability to quench singlet oxygen and scavenge for free radicals. The above-identified DiMascio publication reported the following ability of carotenoids to quench singlet oxygen:

astaxanthin > canthaxathin > β-carotene > zeaxanthin > lutein > > α-tocopherol.

The above-identified Miki publication reported the following ability of carotenoids to scavenge for free radical species:

astaxanthin > zeaxanthin > canthaxanthin > lutein > β-carotene > α-tocopherol.

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Therefore minor structural differences affect the ability of a carotenoid to act as an antioxidant in general, and more particularly to act as a singlet oxygen quencher or a free radical scavenger.

Canthaxanthin, which is a strong antioxidant and which has a structure similar to zeaxanthin and lutein, has the ability to cross the blood-retinal brain barrier. However, canthaxanthin accumulates in the retina to form crystals. Astaxanthin also has a structure similar to canthaxanthin, zeaxanthin and lutein. Astaxanthin also is a stronger antioxidant than canthaxanthin, zeaxanthin and lutein. Surprisingly, astaxanthin, which is not present in human plasma, was found to have the ability to cross the blood-retinal brain barrier (unlike β-carotene), but does not form crystals in the retina and does not otherwise adversely affect the eye (unlike canthaxanthin).

Astaxanthin, or 3,3'-dihydroxy-β,β-carotene-4,4'-dione, is an abundant, naturally-occurring compound. Astaxanthin is the major pigment of crustaceans, and is the most widely distributed xanthophyll in the animal kingdom.

Astaxanthin is a lipid-soluble pigment primarily used for pigmenting cultured fish, like salmon, which must ingest astaxanthin to yield consumer-acceptable pink-colored salmon muscle. Astaxanthin also is an antioxidant which is about 100 to about 1000 times more effective than α-tocopherol.

The prime source of astaxanthin is shells of crustaceans, like krill. Astaxanthin also is available synthetically. Either the naturally-occurring or the synthetic astaxanthin can be used in the composition and method of the present invention.

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In particular, astaxanthin is presently available in three forms: (1) natural astaxanthin extracted from krill, available from Itano Refrigerated Food Co., Tokushima, Japan; (2) synthetic asta-
5   xanthin available from Itano Refrigerated Food Co.; and (3) astaxanthin extracted from Phaffia Rhodozyma yeast, available from Universal Food Co., Milwaukee, Wisconsin. However, astaxanthin in any available form would be used in the method of the present invention.
10   In the following experiments, the astaxanthin extracted from krill was used as received. The synthetic astaxanthin first was mixed with soy bean oil before use. The yeast-based astaxanthin was extracted with acetone to enrich the astaxanthin content. The
15   astaxanthin-rich extract then was mixed with soy bean oil before use.

As previously stated, the retinal pigment epithelium protects the retina by providing a blood-retinal brain barrier. The barrier excludes plasma
20   constituents that are potentially harmful to the retina. As also previously stated, the blood-retinal brain barrier only permits lutein and zeaxanthin to enter the retina, and excludes other carotenoids present in human serum, including β-carotene which is
25   the most abundant carotenoid in human serum. There-fore, an experiment was performed to determine whether astaxanthin, which is not a component of human plasma, has the ability to cross the blood-retinal brain barrier.

30   To determine whether astaxanthin has the ability to cross the blood-retinal brain barrier and concentrate in the retina of a mammal, an astaxanthin solution was prepared by suspending 0.15 g (grams) of AST-5000, an astaxanthin extracted from krill and

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available from Itano Refrigerated Food Co., in 1.0 mL
(milliliters) of a 1.0% by weight aqueous solution of
TWEEN-80 (polyoxyethylene sorbitan monooleate, avail-
able from Sigma Chemical Co., St. Louis Mo.). The
5   resulting astaxanthin solution (1.5 mL) was injected
intraperitoneally into six (6) rats. After euthaniz-
ing the rats, the retinas were examined for the
presence of astaxanthin.   After six consecutive
intraperitoneal injections of astaxanthin at twelve
10   hour intervals and at a dose of about 37.5 mg/kg of
body weight provided an average concentration of
astaxanthin in the retina of about 0.17 μg/mg (micro-
grams per milligram) wet tissue 6 hours after the last
injection.

15   Astaxanthin is not a naturally-occurring
constituent in the retina. Therefore, the presence of
such a significant amount of astaxanthin in the retina
illustrates the ability of astaxanthin to readily
cross the blood-retinal brain barrier into the retina.

20   The ability of astaxanthin to ameliorate eye
damage caused by photic injury was demonstrated as
follows:

             Light-induced photoreceptor degeneration was
conducted according to the protocol reported in Z. Li
25   et al., Current Eye Res., 10, pp. 133-44 (1991),
incorporated herein by reference. Briefly, seven 35
to 40-day old male albino Lewis rats (Harlan, India-
napolis, IN) were fed a normal diet and placed on a
twelve hour cycle of light (uniform illuminance of
30   53.8 lux) and darkness for 14 days. Four rats then
were administered four intraperitoneal injections of
a suspension of 0.15 g of AST-5000 (astaxanthin) in
1.0 mL of aqueous 1.0% TWEEN-80. The dose of each
injection was 37.5 mg astaxanthin/kg of body weight

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per injection. The injections were administered at 12 hour intervals starting at 24 hours before light exposure. Three control rats were injected with 1.0 mL of an aqueous 1.0% TWEEN-80 solution. All seven rats were exposed to 180-200 ft-candle (1800 to 2000 lux), green-filtered fluorescent light (490-580 nm) (Plexiglass No. 2092, Polycast technology, Stamford, CT) for 24 hours after one day of total dark adaptation. All seven rats were kept in the dark for a two-day recovery period, then euthanized with an overdose of pentobarbital. Both eyes of each rat were enucleated for histopathologic and morphometric studies.

The enucleated eyes of each rat were fixed in a 0.1M phosphate-buffered 4% formaldehyde and 1% glutaraldehyde solution (by weight). The anterior segment of each eye was removed, and the posterior segment was divided into the superior (S), nasal (N), inferior (I), and temporal (T) quadrants. Tissue samples from each quadrant then were osmicated (i.e., treated with osmic acid), dehydrated in a graded series of alcohols, and embedded in an epoxy resin. Morphologic and morphometric studies were performed on 1 μm (micrometer) sections stained with Mallory's azure II-methylene blue.

A quantitative determination of photoreceptor cell degeneration was made by measuring the thickness of the outer nuclear layer (ONL) of the retina by a masked observer, as described in J. Michon et al., Invest. Ophthalmol. Vis. Sci., 32, pp. 280-84 (1991), incorporated herein by reference. The measurements were made with an image processing system wherein the stained retinal sections were projected onto a digitizing pad coupled to a microcomputer. The

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measurements were made from the posterior pole to the
ora serrata on two sections.

The bar graphs of FIG. 1 summarize the
results of the photic injury test. From FIG. 1, for
each quadrant and for the retina as whole, the ONL
thickness of the retina was greater for rats receiving
the astaxanthin injection. The astaxanthin therefore
protected the photoreceptors in each of the four eye
quadrants, and in the whole eye as well. For example,
the ONL for the whole eye of rats receiving the
astaxanthin was about 42 μm thick. The ONL of the
whole eye of the control rats was about 32 μm thick.
For comparison, in the absence of a photic injury, the
ONL of a normal eye is about 45 μm thick. FIG. 1
therefore shows that the administration of astaxanthin
provides significant protection to photoreceptor cells
from photic injury.

The ability of astaxanthin to ameliorate
injury to neurons after retinal ischemic insult and
subsequent reperfusion was demonstrated as follows:

Two 45 to 50-day old male albino Lewis rats
(Harlan, Indianapolis, IN) were administered three
intraperitoneal injections of a suspension of 0.15 g
of AST-5000 (astaxanthin) in 1.0 mL of 1.0% aqueous
TWEEN-80 (i.e., a dose 37.5 mg/kg/injection). Two
other albino rats were injected with 1.0 mL solution
of 1.0% aqueous TWEEN-80 as a control. The injections
were administered at 12 hour intervals starting at 24
hours before retinal ischemic insult. Two additional
rats were used as untreated controls.

Four rats were anesthetized with an intra-
peritoneal injection of chloral hydrate (400 mg/kg).
Then, bilateral ischemic insult was induced by elevat-
ing intraocular pressure (IOP) through an infusion

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cannula to the anterior chamber. The IOP was main-
tained at 110 mm (millimeter) Hg for 60 minutes.
Then, the cannulas were removed, and reperfusion of
ocular vessels was established by fundus examination.

5   All four rats were euthanized 7 days after reperfusion
with an overdoes of pentobarbital. Both eyes of each
rat were enucleated and fixed in a 0.1M phosphate
buffer and 1% glutaraldehyde and 4% formaldehyde
solution (by weight).

10   After the anterior segment of each eye was
removed, the posterior segment of each fixed enucleat-
ed eye was divided into the superior (S), temporal
(T), inferior (I) and nasal (N) quadrants. A strip
from each quadrant was sampled, processed and embedded

15   in epoxy resin. Morphologic and morphometric evalua-
tions were performed on 1 μm sections stained with
methylene blue. The degree of ischemic insult was
evaluated by measuring the average thickness (IRT)
between the internal limiting membrane (ILM) to the

20   interface of the outer plexiform layer (OPL) and the
outer nuclear layer (ONL). The measurements were
taken on the projected image of the stained retinal
sections with the aid of image processing. Measure-
ments from all quadrants of the retina were averaged

25   to obtain a mean value for IRT per eye.
The bar graph of FIG. 2 summarizes the
results of the ischemic insult test. From FIG. 2, it
is observed that the average IRT of the retinae of
rats treated with astaxanthin was greater than the IRT

30   of the control rats, seven days after retinal ischemic
insult.
The IRT for rats treated with astaxanthin
was about 70 μm. The IRT for the control rats was
about 62 μm. for comparison, the normal IRT for a rat

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that was not subjected to retinal ischemic insult was
120 μm. The astaxanthin-treated rats, having an IRT
about 10 μm thicker than the untreated rats, demon-
strated that astaxanthin provided statistically-
significant protection to the ganglion cells and other
neuronal elements of the inner retina from ischemic
insult.

In addition to tests using astaxanthin
obtained by the extraction of krill, tests also were
performed using astaxanthin extracted from Phaffia
Rhodozyma. The astaxanthin was extracted using a
modification of the procedure disclosed by E.A.
Johnson et al., App. Environ. Microbiol., 35, pp.
1155-59 (1978), incorporated herein by reference. One
hundred grams of dried Phaffia Rhodozyma powder
(Universal Food Co., Milwaukee, WI.) was mixed and
ground with 200 ml water containing 70 mg of butylated
hydroxytoluene (BHT). The slurry was extracted by
adding 2 L of acetone containing 0.7 gram BHT. The
resulting mixture was stirred at room temperature for
one hour, then centrifuged at 3,110 g for 10 minutes.
The supernatant liquid was separated from the solid
material, then evaporated with a rotary evaporator.
The residue then was extracted again with 1 L acetone
using the same procedure as the first extraction. The
residue of the acetone extractions then was dissolved
in 100 ml acetone containing 35 mg of BHT. The
resulting suspension was air dried and stored at -20C°
until use. The astaxanthin content of the extract was
about 10% by weight (by high pressure liquid chroma-
tography).

Photic injury was induced in 35 to 40 day-
old male albino Lewis rats (Harlan, Indianapolis, IN)
fed a normal diet. The rats were subjected to a 12-

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hour cycle of light (uniform illuminance of 53.8 lux)
and darkness for 14 days. The animals then were given
a daily oral feeding of either: (1) the extracted
astaxanthin in 1.0 mL of soy bean oil at a dose of 80
5   mg astaxanthin/kg/day or (2) 1.0 mL of soy bean oil as
a control, starting nine days before light exposure
and continuing for eleven days. A group of eighteen
rats was fed the extracted astaxanthin, and a second
group of another eighteen rats was fed the soy bean
10   oil control. All thirty-six rats were exposed to 180-
200 ft-candle (1800 to 2000 lux) green filtered
fluorescent light (490-580 nm) (Plexiglass No. 2092,
Polycast technology, Stamford, CT) for 24 hours after
one day of total dark adaptation. All thirty-six rats
15   were kept in the dark for a 6 hour, 6 day, or 13 day
recovery periods, then euthanized with an overdose of
pentobarbital. One eye of each rat was enucleated for
histopathologic and morphometric studies. The second
eye of each rat was used for biochemical evaluation of
20   rhodopsin levels.
        Therefore, one enucleated eye of each rat
was fixed in 0.1 M phosphate-buffered 4% formaldehyde
and 1% glutaraldehyde solution (by weight). The
anterior segment of each eye was removed, and the
25   posterior segment was divided into the superior (S),
nasal (N), inferior (I), and temporal (T) quadrants.
Tissue samples from each quadrant then were osmicated,
dehydrated in a graded series of alcohols, and embed-
ded in an epoxy resin. Morphologic and morphometric
30   studies were performed on 1 μm sections stained with
Mallory's azure II-methylene blue.
        A quantitative determination of photorecep-
tor cell injury was made by measuring the thickness of
the outer nuclear layer (ONL) of the retina by a

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masked observer, as described in the Michon et al. publication incorporated herein by reference. The measurements were made with an image processing system wherein stained retinal sections were projected onto a digitizing pad coupled to a microcomputer. The measurements were made from the posterior pole to the ora serrata on two sections per quadrant.

The second enucleated eye of each rat was used to estimate the retinal rhodopsin levels in accordance with the published method in Z. Li et al., Current Eye Res., 10, pp. 133-44 (1991), incorporated herein by reference. Rhodopsin is a light-sensitive protein found in the rods of the retina in the eye. A decrease in rhodopsin pigment level is indicative of photic injury to the eye. A retina that has not been subjected to photic injury has a rhodopsin level of about 2 to about 2.25 nmol (nanomoles) per eye.

FIG. 3 summarizes the results of this photic injury test. The plots in FIG. 3 show that the rhodopsin level in the retinas of control rats continues to fall for six days following the photic injury, then the rhodopsin level rises. The rhodopsin level of control rats is about 0.75 nmol six hours after photic injury, drops to about 0.5 nmol six days after photic injury, then improves to about 0.8-0.85 nmol thirteen days after photic injury.

In contrast, the astaxanthin-treated rats had a rhodopsin level of about 1.15-1.2 nmol six hours after photic injury. In addition, the rhodopsin level did not decrease over the next six days, but increased to about 1.25 nmol then remained essentially constant through day thirteen after photic injury.

Therefore, astaxanthin not only protected the photoreceptor cells from photic injury (i.e.,

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greater level of rhodopsin six hours after photic
injury) but also ameliorated the effects of the photic
injury because rhodopsin levels did not fall for a
period of six days after the photic injury, but
5   increased.

A similar test was performed wherein β-
carotene was injected into rats, and rhodopsin levels
in the retinae of the euthanized rats were determined.
Rats were given four intraperitoneal injections of β-
10   carotene at a dose of 35 kg/mg. A control group of
rats also was used. After exposure to continuous
green-filtered fluorescent light with an intensity of
220-250 foot-candles for 24 hours, the rhodopsin level
was measured as an indication of photic injury. The
15   β-carotene-treated rats had rhodopsin levels of 1.18,
0.20, and 0.55 nmol, six hours, six days and thirteen
days, respectively, after photic injury. The results
show that astaxanthin is more effective than β-caro-
tene. It is hypothesized, but not relied upon herein,
20   that these comparative results between astaxanthin and
β-carotene is attributed to the antioxidant properties
of astaxanthin and the ability of astaxanthin to cross
the blood-retinal barrier.

The tests using β-carotene show that six
25   hours after light injury there was considerable loss
of photoreceptor nuclei in both the control and
treated rats. Six to fourteen days after light
exposure there was a difference between the control
animals and the treated animals, with the treated
30   animals being less affected. The treated rats demon-
strated better preservation of the photoreceptor
nuclei and retinal pigment epithelium in all quad-
rants. Beta-carotene therefore ameliorated photic
injury in rat retina. However, β-carotene has diffi-

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culty crossing the blood-retinal brain barrier. Astaxanthin both crosses the blood-retinal brain barrier more easily than β-carotene, and has not demonstrated a tendency to form crystals in the retina.

FIG. 4 also summarizes the results of the above-described photic injury test. FIG. 4 includes plots of the average thickness of the ONL over a period of thirteen days after the photic injury. Rats fed astaxanthin had photic damage ameliorated compared to the control rats. The rats fed astaxanthin did not exhibit as large a decrease in ONL six days after photic injury, and exhibited a greater ONL, by about 5μm, thirteen days after photic injury. The tests illustrated in FIGS. 3 and 4 also show that asta-xanthin can be administered orally, as well as intraperitoneally to ameliorate eye damage.

The in vivo test results illustrated in FIGS. 1-4 show the ability of astaxanthin to protect neurons and neuronal elements of the inner and outer retina from photoreceptor cell degeneration in the retina and from ischemic and intraocular pressure-related injury to neurons of the inner layers of retina. The tests have shown that astaxanthin can protect or rescue neurons and other cell types, and that astaxanthin is an effective therapeutic agent to ameliorate photoreceptor degeneration and ischemic damage to neurons of the retina. In addition, asta-xanthin has a protective effect on the central nervous system in general, especially damage to the brain and spinal cord caused by free radicals.

The above tests show that the administration of a therapeutically-effective amount of astaxanthin to an individual prevents, retards and/or ameliorates

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damage to the central nervous system, and especially to the eye, resulting from disease or injury. The astaxanthin is administered to the individual in doses of about 5 to about 500 mg (milligram) astaxanthin per kilogram (kg) of body weight. Preferably, the astaxanthin dose is about 10 to about 200 mg astaxanthin per kg of body weight, and to achieve the full advantage of the present invention, the astaxanthin dose is about 25 to about 150 mg astaxanthin/kg of body weight. The optimal astaxanthin dose can be determined by a person skilled in the art after considering factors such as the disease or injury to be treated, the severity of the central nervous system damage, and the route of administration (i.e., oral, topical or parenterally). The astaxanthin doses can be administered daily or in accordance with a regimen determined by a person skilled in the art, with the length of treatment depending upon the severity and nature of the injury to the central nervous system.

The astaxanthin can be administered to an individual parenterally, orally or topically. When administered orally, the astaxanthin can be, for example, in the form of a liquid preparation, a tablet or as a component of food. When applied topically, the astaxanthin can be, for example, in the form of an eye drop composition for direct application to the eyes.

The administration of astaxanthin to an individual suffering from an eye injury or disease, such as free radical-induced injury, beneficiates the vision of the individual by rescuing further photoreceptor cells from damage destruction. The free radical-induced damage can be attributed to light-induced injury or to injury resulting from an ischemic

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insult and subsequent reperfusion or neurodegenerative diseases. The administration of astaxanthin also helps prevent and retard photic injury in addition to ameliorating photic injury.

The administration of astaxanthin ameliorates photoreceptor cell damage that is light induced, and ameliorates ganglion cell damage that is induced by ischemic insult and subsequent reperfusion. The administration of astaxanthin also retards the progress of degenerative eye diseases and beneficiates the vision of individuals suffering from a degenerative eye disease, such as age-related macular degeneration.

The administration of astaxanthin also provides a method of treating ischemic retinal diseases, such as diabetic retinopathy, cystoid macular edema, central retinal arterial occlusion, central retinal venous occlusion and glaucoma. In addition, astaxanthin administration is useful in treating inflammatory diseases of the eye such as retinitis, uveitis, iritis, keratitis and scleritis wherein free radicals are produced in abundance. These ischemic retinal diseases and inflammatory diseases of the eye are free radical related. Therefore, the antioxidant properties of astaxanthin, coupled with the ability of astaxanthin to cross the blood-retinal brain barrier, lack of toxicity of astaxanthin, and the lack of adverse side effects associated with astaxanthin, make astaxanthin a useful compound to treat or prevent such free radical-related diseases.

Because astaxanthin is a highly-effective antioxidant and ameliorates free radical-induced eye damage, the administration of astaxanthin also provides a method of treating free radical-induced disease or injury to the central nervous system in

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general.   For example, a therapeutically-effective
amount of astaxanthin can be administered to stroke
victims to ameliorate the ischemic insult-related
injury attributed to the stroke. Astaxanthin also can
5   be administered to individuals suffering from a
traumatic injury to the spinal cord which leads to
free radical-induced damage.

Investigators have long searched for effec-
tive antioxidants that can ameliorate neuronal damage.
10   A suitable antioxidant must have the ability to cross
the blood-retinal brain barrier, and must have a low
toxicity. Of the several antioxidants tested to date,
the antioxidant either could not effectively pass the
blood-retinal brain barrier (e.g., β-carotene) or are
15   toxic or exhibited adverse side effects (e.g., cantha-
xanthin). Astaxanthin is a natural, nontoxic product,
and to date has not been shown to be toxic or exhibit
adverse side effects even after the administration of
large doses of astaxanthin for prolonged time periods.
20   For example, astaxanthin has not demonstrated the
disadvantageous side effects of closely-structurally
related canthaxanthin and β-carotene with respect to
forming crystals in the retina or decreasing adapta-
tion to the dark.

25   Because no effective and nontoxic compound
has been found for preserving neurons from various
types of injury or disease, there is no established
medical treatment for diseases such as age-related
macular degeneration. The only active therapy is
30   administration of β-carotene and ascorbic acid.
However, β-carotene cannot effectively cross the
blood-retinal brain barrier. Ascorbic acid is natu-
rally present in the retina. Surprisingly, asta-
xanthin is a strong antioxidant and can effectively

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cross the blood-retinal brain barrier to serve in the
treatment for age-related macular degeneration. The
finding that the administration of astaxanthin also
protects photoreceptors from degeneration also pro-
vides a method of treating diseases and injuries to
the central nervous system which heretofore have been
unavailable.

Obviously, may modifications and variations
of the invention as hereinbefore set forth can be made
without departing from the spirit and scope thereof
and therefore only such limitations should be imposed
as are indicated by the appended claims.

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CLAIMS

1.   A method of beneficiating the vision of
an individual suffering from eye damage or eye dis-
ease, said method comprising administering a therapeu-
tically-effective amount of astaxanthin to the indi-
vidual.

2.   The method of claim 1 wherein the
astaxanthin is administered parenterally.

3.   The method of claim 1 wherein the
astaxanthin is administered orally.

4.   The method of claim 1 wherein the
astaxanthin is administered topically.

5.   The method of claim 1 wherein the
astaxanthin is administered in the amount of about 5
to about 500 milligrams per kilogram of body weight.

6.   The method of claim 1 wherein the
astaxanthin is administered in the amount of about 10
to about 200 milligrams per kilogram of body weight.

7.   The method of claim 1 wherein the
astaxanthin is administered in the amount of about 25
to about 150 milligrams per kilogram of body weight.

8.   The method of claim 1 wherein the eye
damage comprises a free radical-induced eye damage.

9.   The method of claim 1 wherein the eye
damage comprises a light-induced eye damage.

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10. The method of claim 1 wherein the eye damage comprises photoreceptor cell eye damage or damage to neurons of inner retinal layers.

11. The method of claim 1 wherein the eye damage comprises ganglion cell eye damage.

12. The method of claim 1 wherein the eye disease comprises age-related macular degeneration.

13. A method of reducing free radical-induced eye injury comprising administering a therapeutically-effective amount of astaxanthin to an individual.

14. A method of ameliorating neuronal damage to a retina comprising administering a therapeutically-effective amount of astaxanthin to an individual.

15. The method of claim 14 wherein the neuronal damage comprises photic injury to the retina or intraocular pressure-related insult to the retina.

16. The method of claim 14 wherein the neuronal damage comprises ischemic insult to the retina or intraocular pressure-related insult to the retina.

17. A method of treating age-related macular degeneration comprising administering a therapeutically-effective amount of astaxanthin to an individual.

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18. A method of treating ischemic or intraocular pressure-related retinal diseases comprising administering a therapeutically-effective amount of astaxanthin to an individual.

19. The method of claim 18 wherein the ischemic retinal disease is selected from the group consisting of diabetic retinopathy, cystoid macular edema, central retinal arterial occlusion, central retinal venous occlusion, and glaucoma.

20. A method of treating an inflammatory disease of the eye comprising administering a therapeutically-effective amount of astaxanthin to an individual.

21. The method of claim 20 wherein the inflammatory disease is selected from the group consisting of retinitis, uveitis, iritis, keratitis, and scleritis.

22. A method of treating a free radical-induced injury to a central nervous system, said method comprising administering a therapeutically-effective amount of astaxanthin to an individual.

23. The method of claim 22 wherein the central nervous system comprises a brain, a spinal cord and an eye.

24. The method of claim 23 wherein the free radical-induced injury comprises a traumatic injury or an ischemic injury.

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25. The method of claim 24 wherein the ischemic injury comprises a stroke.

26. The method of claim 24 wherein the traumatic injury comprises a spinal cord injury.

27. A method of retarding a degenerative eye disease, said method comprising administering a therapeutically-effective amount of astaxanthin to an individual.

28. A method of retarding a degenerative central nervous system disease, said method comprising administering a therapeutically-effective amount of astaxanthin to an individual.

Int   ional Application No

PCT/US 95/14502

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   A61K31/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6   A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	C.R.ACAD.SC.PARIS, vol. 270, no. 9, 1970 pages 1282-1283, XP 000563608 'Biochimie de la vision' see page 1282, paragraph 1 see page 1283, paragraph 2 ---	1-12,27
X,P	WO,A,95 00130 (HOWARD FOUNDATION) 5 January 1995 see page 4, paragraph 1-2 see page 7, line 15 see page 23, line 23 see claims 5,10 --- -/--	1-13, 22-28

[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 March 1996	22. 03.96
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Authorized officer Gerli, P

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PHYSIOL.CHEM.PHYS.MED.NMR, vol. 22, no. 1, 1990 pages 27-38, XP 000563607 'Inhibition of oxidative injury of biological membranes by astaxanthin' see the whole document ---	1-28
Y	J.PHOTOCHEM.PHOTOBIOL.B BIOL., vol. 26, no. 3, 1994 pages 283-5, XP 000563681 'Dietary carotenoids protect human cells from damage' see the whole document -----	1-28

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Box I   Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:
   Remark: Although claims 1-28 are directed to a method of treatment of

   (diagnostic method practised on) the human/animal body the search has been
   carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such
   an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II   Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all
   searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment
   of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report
   covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is
   restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest   [ ] The additional search fees were accompanied by the applicant's protest.

                               [ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1992)

Int. 'onal Application No

PCI/US 95/14502

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9500130	05-01-95	AU-B-	7005694	17-01-95
		GB-A-	2280110	25-01-95

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